UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: September 4, 2015

Subject: Efficacy Review for SaniDate 15.0
EPA File Symbol 70299-EA (DP Barcode 427134)

From: Alison Clune
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

Thru: Mark Perry, Team Leader
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To: Julie Chao / Seiichi Murasaki, Team 33
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: BioSafe Systems, LLC
22 Meadow St.
East Hartford, CT 06108

Formulation from the Label:

Active Ingredient(s)	% by wt.
Hydrogen peroxide	10.0%
Peroxyacetic acid	15.0%
Other Ingredients	75.0%
Total	100.0%

I BACKGROUND

The product, SaniDate 15.0 (EPA File Symbol 70299-EA), is a new product. The applicant requested to register the product as a hard surface sanitizer for commercial indoor food and non-food uses. The applicant is submitting efficacy data from studies conducted at Bioscience Laboratories, Inc., 1755 South 19th Avenue, Bozeman, Montana 59718.

This data package contained a letter dated April 10, 2015 from the applicant to EPA, EPA Form 8570 (Confidential Statement of Formula), 2 efficacy studies (MRID 49613903 and 49613904), and the proposed label. Statements of No Data Confidentiality Claim, Good

Laboratory Practice Compliance Statements, Quality Assurance Statements, and Certificates of Analysis of the concentration of the active ingredient for each product lot were included with each study.

II USE DIRECTIONS

The proposed product label indicates that it is designed for sanitizing hard, non-porous food contact surfaces in commercial food production facilities including federally inspected meat, seafood and poultry facilities; pet food plants; milk and dairy product processing/packing plants; wineries, breweries, and beverage plants; fruit, nut, and vegetable packing and processing facilities; and egg processing plants. In these facilities, the proposed label indicates that the product may be used to sanitize conveyors, peelers, slicers, and saws; milking equipment; casing or shell eggs; hatching eggs; hatchery rooms, poultry houses, and livestock buildings; packinghouses; harvesting and field equipment and transportation vehicles; and as a final sanitizing bottle rinse. The proposed product label also indicates that the product may be used to sanitize hard, non-porous, non-food contact surfaces such as ceilings, drains, floors, and walls.

Directions on the proposed label provide the following information regarding preparation and use of the product:

Sanitization of Hard, Non-porous Food Contact Surfaces, Pathogenic Organisms:
Clean equipment immediately after use:

1. Remove gross particulate matter with a warm water flush.
2. Wash equipment with detergent or cleaning solution.
3. Rinse equipment with potable water.
4. Prepare SaniDate 15.0 solution by adding 0.41-0.94 fl. oz. to 5 gallons of potable water. This provides 109-250 ppm peroxyacetic acid and 73-167 ppm of hydrogen peroxide.
5. Fill closed systems with diluted sanitizer solution for a contact time of one (1) minute.
6. For open or not completely closed systems, use a coarse spray, mop/wipe or flood technique to apply the solution to the surface for a contact time of at least one (1) minute. Allow surfaces to drain thoroughly before resuming operation.

Sanitization of Hard, Non-porous, Non-food Contact Surfaces:

1. Remove gross contamination with a cleaner or other suitable detergent and rinse with potable water.
2. Prepare SaniDate 15.0 solution by adding 0.41-0.94 fl. oz. to 5 gallons of potable water. This provides 109-250 ppm peroxyacetic acid and 73-167 ppm of hydrogen peroxide.
3. Apply by wiping, mopping, coarse spray, foam, or flood.
4. Allow a contact time of five (5) minutes.
5. Allow items and/or surfaces to air dry before resuming operation.
6. Do not rinse.

III AGENCY STANDARDS FOR THE PROPOSED CLAIMS

Sanitizing Rinses (For Non-halide chemical products on Previously Cleaned, Food Contact Surfaces):

For sanitizing rinses formulated with quaternary ammonium compounds, chlorinated trisodium phosphate and anionic detergent-acid formulations, the Agency recommends the AOAC International Germicidal and Detergent Sanitizing Action of Disinfectants test. Three samples,

representing three different batches, should be evaluated for efficacy against both *Escherichia coli* (ATCC 11229) and *Staphylococcus aureus* (ATCC 6538). When claims are made for the effectiveness of the product in hard water, all data should be developed at the hard water tolerance claimed. Acceptable results should demonstrate a ≥99.999% reduction in the number of each test microorganism within 30 seconds. The AOAC method states that the numbers control must fall between 7.0-8.0 logs for both organisms for a valid test. The results should be reported according to the actual count and percentage reduction over the control.

Sanitizers (For Food Contact Surfaces, Additional Bacteria):

There are cases where an applicant requests to make claims of effectiveness against additional microorganisms for a product that is to be used as a sanitizer for non-food contact surfaces. Confirmatory test standards would apply. Therefore, 2 product samples, representing 2 different product lots, should be tested against each additional microorganism. Acceptable results should demonstrate a ≥99.999% reduction in the number of each test microorganism within 30 seconds. The AOAC method states that the numbers control must fall between 7.0-8.0 logs for each organism for a valid test. The results should be reported according to the actual count and percentage reduction over the control.

Sanitizers for Non-Food Contact Surfaces:

The effectiveness of sanitizers for non-food contact surfaces must be supported by data that show that the product will substantially reduce the numbers of test bacteria on a treated surface. For liquid, water soluble powder, and spray products, this is accomplished by the ASTM method E1153 (Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate, Hard, Non-porous Non-Food Contact Surfaces). The test surface(s) should represent the type(s) of surfaces recommended for treatment on the label, i.e., porous or non-porous. Tests should be performed with each of 3 product samples, representing 3 different product lots, all tested at or below the lower certified limit(s) of the active ingredient(s), against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048). Five test carriers and 3 control carriers should be evaluated as described in the method with at least a 7.5×10^5 carrier population control count. Results must show a bacterial reduction of at least 99.9% over the parallel control within 5 minutes.

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. **MRID 49613903 "An Evaluation of the Bactericidal Efficacy of One Test Formulation When Used as a Food Contact Sanitizer", by Jessica J. McDonnell-Philipp. Bacteria: *Campylobacter jejuni, Escherichia coli, Escherichia coli O157:H7, Listeria monocytogenes, Salmonella enterica, Staphylococcus aureus.* Study conducted at Bioscience Laboratories, Inc. Study completion date: 3/17/15. Laboratory Study Identification Number: 140525-204.**

This study was conducted against *Campylobacter jejuni* subsp. *jejuni* (ATCC 29428), *Escherichia coli* (ATCC 11229), *Escherichia coli* serotype O157:H7 (ATCC 35150), *Listeria monocytogenes* serotype 2 (ATCC 19112), *Salmonella enterica* subsp. *enterica* serovar Choleraesuis (ATCC 10708), and *Staphylococcus aureus* subsp. *aureus* (ATCC 6538). Three lots (SD15081614A, SD15081614B, and SD15081614E) of the product, SaniDate 15.0, were tested using the Bioscience Laboratories, Inc. protocol "An Evaluation of the Bactericidal Efficacy of One Test Formulation when used as a Food-Contact Sanitizer", protocol number 140525-204 which follows AOAC Official Method 960.09. The three batches of the test substance were diluted at or below the lower certified limits of the active ingredients with 400 ppm AOAC synthetic hard

water. To prepare all test organisms except *Campylobacter jejuni*, stock organism was suspended in phosphate buffer dilution water (PBDW), inoculated on Nutrient Agar A slants, and incubated at 36 ± 1°C for 24 ± 2 hours. One daily transfer of each culture to fresh Nutrient Agar A slants was performed and incubated at 36 ± 1°C for 24 ± 2 hours. To initiate the final test cultures, growth on the Nutrient Agar A slants was suspended in 5 mL PBDW and transferred to flasks containing 99 mL PBDW. This mixture was plated in 0.2 mL aliquots on at least 5 Nutrient Agar B plates and incubated at 36 ± 1°C for 24 ± 2 hours. Just before testing, growth on the Nutrient Agar B plates was suspended in 5 mL phosphate buffered saline with 0.1% Tween 80 and pooled in a sterile vessel. PBDW was added as necessary to the *Escherichia coli* and *Staphylococcus aureus* cultures to achieve a concentration of $10^9 - 10^{10}$ CFU/mL. To prepare *Campylobacter jejuni*, stock organism was suspended in 0.9% sodium chloride irrigation USP (SCI), inoculated on Tryptic Soy Agar with 5% sheep blood (SBA), and incubated microaerophilically at 36 ± 1°C for 3 days. Growth on the SBA plates was suspended in SCI, spread plated on fresh SBA plates, and incubated microaerophilically at 36 ± 1°C for 3 days. Just before testing, the growth from the final SBA plates was suspended in PBDW, pooled, and adjusted with PBDW and/or centrifuged as necessary to achieve the appropriate test concentration. *Escherichia coli* and *Staphylococcus aureus* were tested against all three product lots, while the remaining organisms were tested against only two product lots (SD15081614A and SD15081614B). For each product lot versus test organism, 1.0 mL of test culture was added to a flask containing 99.0 mL of the test substance, swirling the liquid in the flask to prevent pooling. After the 30 second contact time, 1 mL of the liquid containing test substance and test organism was removed and neutralized in 9 mL neutralization broth. The neutralized mixture was vortex mixed and pour plated in quadruplicate in volumes of 1 mL and 0.1 mL. Samples from testing against *Campylobacter jejuni* were plated on SBA and incubated for 36 ± 1°C for 5 days. Samples from testing against all other cultures were plated on Tryptone Glucose Extract Agar and incubated at 36 ± 1°C for 24-30 hours. Following incubation, colonies were counted manually. Controls included purity, sterility, neutralization confirmation and toxicity (both product lots), and numbers controls.

Note: A protocol deviation occurred when the *Escherichia coli* and *Staphylococcus aureus* cultures were not vacuum filtered through Whatman No. 2 filter paper to remove agar chunks prior to use in testing. However no chunks were observed in the test organism suspension at the time it was pooled for testing.

2. **MRID 49613904 "An Evaluation of the Sanitizing Efficacy of One Test Formulation For Use on Non-Food Contact Surfaces", by Lisa Lehman. Bacteria: *Klebsiella pneumoniae pneumoniae, Staphylococcus aureus* subsp. *aureus*. Study conducted at Bioscience Laboratories, Inc. Study completion date: 2/12/15. Laboratory Study Identification Number: 141111-204.**

This study was conducted against *Klebsiella pneumoniae pneumoniae* (ATCC 4352) and *Staphylococcus aureus* subsp. *aureus* (ATCC 6538). Three lots (SD15081614A, SD15081614B, and SD15081614E) of the product, SaniDate 15.0, were tested using the Bioscience Laboratories, Inc. protocol "An Evaluation of the Sanitizing Efficacy of One Test Formulation for Use on Non-Food Contact Surfaces", protocol number 141111-204, which follows ASTM Standard Test Method E1153-10. The three batches of the test substance were diluted at or below the lower certified limits of the active ingredients with 400 ppm AOAC synthetic hard water. Ten mL tubes of Nutrient Broth were inoculated with lyophilized stock organism and incubated for 24 hours at 35 ± 2°C. Daily transfers of 10 µL of culture into additional 10 mL tubes of Nutrient Broth were incubated at 35 ± 2°C. These broth cultures were subcultured to sufficient tubes containing 10 mL Nutrient Broth and incubated for 48-54 hours. Subcultures were vortex mixed on the day of

testing and allowed to stand 15 minutes before the upper portions of the cultures were pooled in a sterile vessel and mixed. Initial inoculum counts were determined by serially diluting each pooled suspension in PBDW and plating each dilution in duplicate on Nutrient Agar with product neutralizers. Plates were incubated for 48-50 hours at 35 ± 2°C and the resulting colonies were hand counted. At least 15 sterile glass 1" x 1" carriers were inoculated with 0.02 mL of each test organism, which was spread over the carrier, and dried for 20-40 minutes at 30-37°C in an incubator. Carriers were transferred to separate 2 oz. sterile containers and 5.0 mL of test substance was added to each jar, completely covering the carrier. For each test organism, five carriers were treated with each batch of the test substance and 3 carriers were treated with control solution. The carriers were exposed to the test substance for 5 minutes at ambient temperature. At the end of the exposure time, 20 mL of neutralizer was added and the jars were rotated vigorously for at least 1 minute. Duplicate aliquots of 1.00 mL and 0.100 mL of the neutralized solution were pour plated on Nutrient Agar with neutralizers and incubated for 48-50 hours at 35 ± 2°C. Following incubation, colonies were counted manually. Controls included purity, sterility, carrier population, and neutralizaton confirmation (all product batches) controls.

V EFFICACY RESULTS

Food Contact Sanitizing Results

MRID 49613903				
Organism	**Post-exposure Concentration of Test Organism log_{10} CFU/mL (Percent Reduction)**			**Numbers Controls (log_{10} CFU/mL)**
	Lot SD15081614A	**Lot SD15081614B**	**Lot SD15081614E**	
30 second contact time				
Staphylococcus aureus (ATCC 6538)	<1.0000 (>99.999999%)	<1.0000 (>99.999999%)	<1.0000 (>99.999999%)	8.1732
Escherichia coli (ATCC 11229)	<1.0000 (>99.999994%)	<1.0000 (>99.999994%)	<1.0000 (>99.999994%)	7.2535
Escherichia coli O157:H7 (ATCC 35150)	<1.0000 (>99.999987%)	<1.0000 (>99.999987%)	NA	6.8935
Listeria monocytogenes (ATCC 19112)	<1.0000 (>99.999962%)	<1.0000 (>99.999962%)	NA	6.4245
Salmonella enterica (ATCC 10708)	<1.0000 (>99.999990%)	<1.0000 (>99.999990%)	NA	6.9811
Campylobacter jejuni (ATCC 29428)	<1.0000 (>99.999973%)	<1.0000 (>99.999973%)	NA	6.5653

Non-Food Contact Sanitizing Results

MRID 49613904					
Organism	**Geometric Mean CFU/carrier (Percent Reduction)**			**Carrier Population Control (Geometric mean CFU/carrier)**	**Carrier Population Control (Average CFU/carrier)**
	Lot SD15081614A	**Lot SD15081614B**	**Lot SD15081614E**		
5 minute contact time					
Staphylococcus aureus aureus (ATCC 6538)	$<2.50 \times 10^1$ (>99.9%)	$<2.50 \times 10^1$ (>99.9%)	$<2.50 \times 10^1$ (>99.9%)	1.98×10^6	8.71×10^6
Klebsiella pneumoniae pneumoniae (ATCC 4352)	$<2.50 \times 10^1$ (>99.9%)	$<2.50 \times 10^1$ (>99.9%)	$<2.50 \times 10^1$ (>99.9%)	6.64×10^5	1.34×10^6

VI CONCLUSIONS

1. The submitted efficacy data **supports** the use of the product, SaniDate 15.0, as a sanitizer on hard, non-porous, food contact surfaces when diluted at 0.41 fl. oz. per 5 gallons of water and applied for a 30 second contact time against the following bacteria:

   Staphylococcus aureus (ATCC 6538)
   Escherichia coli (ATCC 11229)

   According to the analysis of the active ingredient concentration for each product batch, the tested dilutions were at or below the lower certified limit of the active ingredient. Acceptable log reduction was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the test organisms.

2. The submitted efficacy data **does not support** the use of the product, SaniDate 15.0, as a sanitizer on hard, non-porous, food contact surfaces when diluted at 0.41 fl. oz. per 5 gallons of water and applied for a 30 second contact time against the following bacteria:

   Escherichia coli O157:H7 (ATCC 35150)
   Listeria monocytogenes (ATCC 19112)
   Salmonella enterica (ATCC 10708)
   Campylobacter jejuni (ATCC 29428)

   In testing against these organisms, the numbers control did not reach the acceptable range of 7.0-8.0 logs for a valid test. Because the tests did not fail, repeat testing of the product against these organisms under the same conditions is allowed in order to produce a valid result.

3. The submitted efficacy data **supports** the use of the product, SaniDate 15.0, as a sanitizer on hard, non-porous, non-food contact surfaces when diluted at 0.41 fl. oz. per 5 gallons of water and applied for a 5 minute contact time against the following bacteria:

   Klebsiella pneumoniae pneumoniae (ATCC 4352)
   Staphylococcus aureus aureus (ATCC 6538)

   According to the analysis of the active ingredient concentration for each product batch, the tested dilutions were at or below the lower certified limit of the active ingredient. Acceptable log reduction was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the test organisms.

VII LABEL

1. The proposed label claims that the product, SaniDate 15.0, when diluted at 0.41-0.94 fl. oz. per 5 gallons of water is an effective sanitizer on hard, non-porous, food contact surfaces against the following bacteria when applied for a 1 minute contact time:

   Staphylococcus aureus (ATCC 6538)
   Escherichia coli (ATCC 11229)

   These claims are **acceptable** as they are supported by the submitted data.

2. The proposed label claims that the product, SaniDate 15.0, when diluted at 0.41-0.94 fl. oz. per 5 gallons of water is an effective sanitizer on hard, non-porous, food contact surfaces against the following bacteria when applied for a 1 minute contact time:

   Escherichia coli O157:H7 (ATCC 35150)
   Listeria monocytogenes (ATCC 19112)
   Salmonella enterica (ATCC 10708)
   Campylobacter jejuni (ATCC 29428)

   These claims are **not acceptable** as they are not supported by the submitted data. These claims must be removed from the product label.

3. The proposed label claims that the product, SaniDate 15.0, when diluted at 0.41-0.94 fl. oz. per 5 gallons of water is an effective sanitizer on hard, non-porous, non-food contact surfaces against the following bacteria when applied for a 5 minute contact time:

   Klebsiella pneumoniae pneumoniae (ATCC 4352)
   Staphylococcus aureus aureus (ATCC 6538)

   These claims are **acceptable** as they are supported by the submitted data.

4. The organisms against which the product, SaniDate 15.0, was successfully tested should be identified by their ATCC numbers at least once on the label.

5. On pages 4 and 6 of the proposed label, "course spray" should be changed to "coarse spray".

6. On page 5 of the proposed label, "MILIKING" should be changed to "MILKING" in the heading "SANITIZING MILKING EQUIPMENT BY CLUSTER DIPPING".

7. On page 5 of the proposed label, under the heading "FOR SANITIZING OF CASING OR SHELL EGGS", the statement "Apply dilute solution as eggs are gathered as a coarse spray or flood." should be removed. Shell eggs intended for food or food products may only be treated with a spray.

8. The treatment of eggs intended for use as hatchlings is considered a drug claim and is regulated by FDA. It is not allowed on FIFRA labels and must be removed from page 5 of the proposed label.

9. On page 5 of the proposed label, under the heading "PACKINGHOUSE SANITIZATION", *Aspergillus versicolor* should be removed from the list of microorganisms against which the product is an effective sanitizer. *Aspergillus versicolor* is of public health significance, and so cannot be listed without the appropriate efficacy data to support this claim.

10. On page 6 of the proposed label, "NON-PORUS" should be changed to "NON-POROUS" in the heading "SANITIZATION OF HARD, NON-POROUS, NON-FOOD CONTACT SURFACES".

11. On page 6 of the proposed label, in the list of non-food contact surfaces that the product is intended to treat "drains" should be removed or qualified such that the claim applies only to the opening of the drain or the grate covering the opening of the drain. Drain sanitizers typically require special testing of the product applied as a foam in the presence of a high organic soil load to demonstrate efficacy. The submitted data does not support a drain sanitizing claim.

12. On page 7 of the proposed label, the heading regarding the control of slime forming bacteria and biofouling in cooling water systems and water features should be changed to limit the claim to "non-public health slime forming bacteria".

13. On page 7 of the proposed label, the term "recreational" should be removed from the heading regarding the control of slime forming bacteria and biofouling in cooling water systems and water features to limit the implication that the product will control public health organisms in swimming pool, hot tub, or other recreational water.

14. On page 8 of the proposed label, the heading regarding microbial control in sewage and wastewater effluents should be changed to limit the claim to "non-public health microbial control".

15. The term "Microbiocide" is inappropriate for a sanitizer product label with public health claims and should be removed from page 11 of the proposed label.

16. The statements "Treats and controls mold and mildew", "Fungicide", and all other claims for the efficacy of the product against fungi, mold and mildew should be removed from the proposed label (pages 11-12). There are no directions for use for the treatment or control of mold and mildew, and data should be submitted to support the use of the product, SaniDate 15.0 as a mildew fungistat or fungicide.

17. On page 12 of the proposed label, the statement "SaniDate 15.0 meets AOAC efficacy standards for hard surface non-food contact sanitizing" is inaccurate. The test method that was used to develop the submitted data for non-food contact surface sanitizing efficacy is published by ASTM International, not AOAC International. The method does not specify efficacy standards.